METHODS OF INCREASING PLATELET AND HEMATOPOIETIC STEM CELL PRODUCTION

This application claims priority to Application Nos. 60/411,779 and 60/411,700 filed on September 18, 2002.

BACKGROUND OF THE INVENTION

Thrombopoietin (TPO), initially cloned as a major regulator of platelet production, plays a pivotal role in hematopoietic stem cell (HSC) biology. Kaushansky et al., Nature, 369:568-571 (1994). Virtually all primitive HSC that display repopulating activity express c-Mpl, the receptor of TPO. Solar et al., Blood, 92:4-10 (1998). TPO alone or in combination with other early acting cytokines, such as stem cell factor (SCF), interleukin 3 (IL-3), or Flt-3 ligand enhance proliferation of primative HSC in vitro. Ku et al., Blood, 87:4544-4551 (1996); Sitnicka et al., Blood, 87:4998-5005 (1996). In vivo studies have confirmed these conclusions. Kimura et al., Proc. Natl. Acad. Sci. U.S.A., 95:1195-1200 (1998). The importance of TPO in stem cell self renewal and expansion was also supported by the clinical observation that mutations of the c-Mpl gene caused congenital amegakaryocytic thrombocytopenia, a disease in which all hematopoietic lineages fail during childhood. Ballmaier et al., Blood, 97:139-146 (2001). It has been found that expansion of HSCs in adult bone marrow is 10 to 20 times less robust in tpo-/- mice following bone marrow transplantation. Exogenously added TPO rescued this defect. Fox et al., J. Clin. Invest., 110:389-394 (2002). These reports indicate that TPO is a major non redundant contributor to self renewal and expansion of HSCs.

Autologous stem cell transplantation (ASCT) is increasingly widely used as a means of reconstituting the bone marrow following the administration of potentially curative, myeloablative, high dose chemotherapy. The basis for this technique is to mobilize HSCs from bone marrow to peripheral blood (using G-CSF +/- priming chemotherapy) from which they are harvested by apheresis. These stem cells, which form a minority of the harvested population, are then capable of reconstituting the bone marrow when reinfused following myeloablative therapy. Stem cells obtained from peripheral blood in this technique appear to be similar to cord blood cells and superior to bone marrow cells in their ability to regenerate bone marrow following myeloablative therapy with time to neutrophil and platelet engraftment of less than 10

days. The most common tumor types in which ASCT is used are myeloma, lymphoma (both Hodgkins Disease and Non-Hodgkins Lymphoma) and Acute Myeloid Leukemia. High dose chemotherapy with ASCT may be increasingly used as first line therapy, particularly in myeloma, but it is also used as salvage therapy following the failure of first line chemotherapy. Such subjects often have been heavily pretreated and thus have bone marrow with impaired hematopoietic potential.

Following the re-infusion of these harvested cells into the subject, there is a period of time during which the subject, e.g., a human patient, is at risk of infection (low neutrophils) and bleeding (low platelets). This period of time varies depending on the number of re-infused stem cells, which in turn depends on the ability to stimulate the expansion of stem cells from bone marrow. Further, some subjects also develop bone marrow failure after an initial period of engraftment.

Stem cell transplantation is also used in an allogeneic setting when peripheral blood stem cells are mobilized and harvested from HLA matched donors. Such allogeneic transplants are less frequently employed than ASCT because of the incidence of graft versus host disease but may be used when it is not possible to obtain sufficient stem cells from the patient. However the use of allogeneic stem cells to obtain partial engraftment in the absence of complete myeloablation (the 'mini transplant') may also offer some therapeutic benefit due to a graft versus tumor effect. Another possible use, currently in an extremely small number of patients is in the field of gene therapy where normal allogeneic bone marrow cells or autologous cells transduced with a normal copy of a defective gene may be curative for some inherited disease caused by single gene defects. Allogeneic transplants are also under investigation as a therapeutic option for autoimmune diseases.

Despite the potential utility and simplicity of ASCT, there are significant limitations to its widespread use beyond the expected period of pancytopenia, for which intensive subject support is required to allow the re-infused cells to resume levels of hematopoiesis sufficient to maintain peripheral blood counts. A significant proportion (up to 40%) of transplanted subjects require prolonged platelet transfusions following transplant (primary failure of engraftment). A smaller group (5-10% in autologous but >20% with allogeneic transplant) develop secondary thrombocytopenia despite initial engraftment, sometimes requiring prolonged transfusions. Failure of engraftment or

delayed engraftment is associated with increased mortality, increased healthcare costs and decreased subject quality of life.

There thus exists a need to increase HSC production in such subjects. Studies have demonstrated that administration of TPO to patients results in mobilization of peripheral blood progenitor cells. One study demonstrated the mobilization of colony forming cells from multiple lineages and CD34+ cells into the peripheral blood following multiple dose administration of TPO in combination with G-CSF. Another study identified a 6 fold increase in circulating CD34+ cells 3-7 days after administration of a single dose of TPO in cancer patients with otherwise normal hematopoiesis. In this study, a stem cell enriched subfraction (CD34+Thy+Lin-) was increased nearly 9 fold and the committed megakaryocytic subfraction (CD34+CD41+CD14-) was increased nearly 15 fold. This study suggests that TPO is capable of mobilizing both self-renewing HSC and committed daughter cells from bone marrow. Although the availability of recombinant TPO (rhTPO) has shown promise in increasing HSC production, a need exists for an improvement in TPO therapy by way of the mode of drug delivery

There thus exists a need for small molecule mimetic compounds of TPO that retain substantially the full agonist activity of TPO, while at the same time permitting various modes of administration.

There also exists a need for small molecule mimetic compounds of TPO having reduced immunogenicity relative to one or more of rhTPO and rhIL-11 as well as improved pharmacokinetic profile relative to one or more of rhTPO and rhIL-11.

SUMMARY OF THE INVENTION

The present invention is directed to a method of increasing HSC production in a subject comprising a step of administering a TPO mimetic compound to the subject. The TPO mimetic compound may be administered to the subject alone or in a pharmaceutically acceptable carrier. The TPO mimetic compound can be employed alone or can be combined with one or more additional TPO mimetic compounds and/or other agents that can enhance stem cell mobilization from bone marrow, including, e.g., G-CSF, SCF, IL-3 and/or Flt-3.

The present invention is thus also directed to a TPO mimetic pharmaceutical composition that comprises an effective amount of a TPO mimetic compound and a pharmaceutically acceptable carrier. An effective amount of a TPO mimetic compound is present when upon administration the TPO mimetic compound enhances expansion of the stem cell population within bone marrow of a subject and/or mobilizes the stem cells into the peripheral circulation of a subject.

The present invention is also directed to a method of providing HSCs to a subject. The method can include the steps of administering a TPO mimetic compound to the subject to enhance expansion of the stem cell population within bone marrow and/or to mobilize the stem cells into the peripheral circulation. Next, the method can include harvesting one or more stem cells from the subject from either the bone marrow or from the peripheral circulation and then transplanting the harvested one or more stem cells into the subject.

The present invention is also directed to a method of providing HSCs from a donor subject to a recipient subject.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a listing of compounds, which can be suitable for use in the method of the present invention.

Fig. 2 is a schematic which represents regulation of platelet and HSC production in accordance with the method of the present invention.

DETAILED DESCRIPTION OF THE INVENTION

The relevant portions of the patent publications and literature cited herein are incorporated by reference herein.

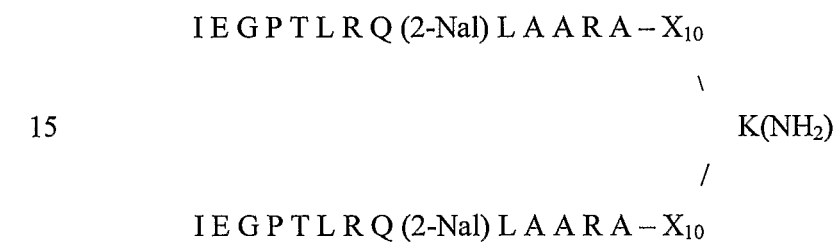
In one embodiment, the present invention is directed to increasing HSC production by administering to a subject a TPO peptide, a TPO mimetic compound, including, but not limited to the compounds set forth in Fig. 1, and PEGylated forms of the compounds set forth in Fig. 1. The methodology that may be employed for PEGylation of the compounds set forth in Fig. 1 is described in U.S. Patent No. 5,869,451.

In an embodiment, the present invention is directed to increasing HSC production by administering to a subject a TPO peptide, as described in corresponding U.S. application serial no. _____ (attorney docket no. 038073-5005 PR), filed August 28, 2003, the entire contents of which are incorporated herein by reference. According to this embodiment, the TPO peptide is a compound having (1) a molecular weight of less than about 5000 daltons, and (2) a binding affinity to TPO receptor as expressed by an IC_{50} of no more than about 100 μM, wherein from zero to all of the –C(O)NH—linkages of the peptides have been replaced by a linkage selected from the group consisting of $–CH_2OC(O)NR—$linkage; a phosphonate linkage; a $–CH_2S(O)_2NR—$linkage; a $CH_2NR—$linkage; a $C(O)NR^6$ linkage; and a –NHC(O)NH—linkage where R is hydrogen or lower alkyl and R^6 is lower alkyl, further wherein the N-terminus of said compound is selected from the group consisting of a $–NRR^1$ group; a –NRC(O)OR group; a $–NRS(O)_2R$ group; a –NHC(O)NHR group; a succinimide group; a benzyloxylcarbonyl-NH group; and a benzyloxycarbonyl-NH group having from 1 to 3 substituents on the phenyl ring selected from the group consisting of lower alkyl, lower alkoxy, chloro and bromo, where R and R^1 are independently selected from the group consisting of hydrogen and lower alkyl, and still further when the C-terminus of the compound has the formula $–C(O)R^2$ where R^2 is selected from the group consisting of hydroxy, lower alkoxy, and $–NR^3R^4$ where R^3 and R^4 are independently selected from the group consisting of hydrogen and lower alkyl and where the nitrogen atom of the $–NR^3R^4$ group can optionally be the amine group of the N-terminus of the peptide so as to form a cyclic peptide, and physiologically acceptable salts thereof.

In a related embodiment, the TPO mimetic peptide comprises a sequence of amino acids $X_9X_8GX_1X_2X_3X_4X_5X_6X_7$, where X_9 is A, C, E, G, I, L, M, P, R, Q, S, T or V; and X_8 is A, C, D, E, K, L, Q, R, S, T or V; and X_6 is a β-(2-napthyl)alanine (referred to herein as "2-Nal") residue. More preferably, X_9 is A or I, and X_8 is D, E or K. Further, X_1 is C, L, M, P, Q or V; X_2 is F, K, L, N, Q, R, S, T or V; X_3 is C, F, I, L, M, R, S, V or W; X_4 is any of the 20 genetically coded L-amino acids; X_5 is A, D, E, G, K, M, Q, R, S, T, V or Y; and X_7 is C, G, I, K, L, M, N, R or V.

A particularly preferred TPO mimetic peptide is I E G P T L R Q (2-Nal) L A A R A.

In another embodiment, the TPO mimetic peptide is dimerized or oligomerized to increase the affinity and/or activity of the compound. An example of such a compound includes:



where X_{10} is a sarcosine or β-alanine residue or a pegylated form of this compound. The pegylated form may include a 20k MPEG residue covalently linked to each N-terminal isoleucine.

One or more TPO mimetic peptides, and in particular PEGylated TPO mimetic peptides (collectively referred to herein as "TPO mimetic compounds" or "TPO mimetic compounds of the invention"), can be used to increase the number of stem cells in bone marrow. Important data supporting the use of a TPO mimetic compound in ASCT is provided by a study performed by Somlo et al., Blood, 93(9):2798-2806 (1999), in which recombinant human thrombopoietin (rhTPO) was able to enhance the mobilization and apheresis yields of CD34+ stem cells in response to G-CSF with consequent reduction in the number of aphereses. Subsequently the engraftment of reinfused cells was also improved in terms of reduced time to ANC>0.5 x 10^9/L and platelet transfusion independence, though this effect did not reach statistical significance in the small sample size used in this pilot study. By increasing the number of stem cells, the total harvest of stem cells from the subject can be significantly

improved. Further, by increasing the number of stem cells harvested from the subject, the number of stem cells available for transplantation back into the subject can also be significantly improved, thereby potentially reducing the time to engraftment (the time during which the subject has insufficient neutrophils and platelets), thus preventing complications.

In addition, the present invention can also reduce the proportion of subjects who are unable to harvest enough cells to proceed with treatment for their primary illness, e.g., chemotherapy and other bone marrow ablative treatments. Furthermore, the proportion of the number of subjects with delayed primary engraftment can also be reduced.

TPO mimetic compounds such as those in Fig. 1 and disclosed herein can be used to increase HSC production. This is accomplished by administering one or more of the compounds to a subject. The compounds set forth in Fig. 1 and disclosed herein, as well as PEGylated forms of the compounds, set forth in Fig. 1 can have reduced immunogenicity relative to one or more of rhTPO and rhIL-11 and can also have an improved pharmacokinetic profile relative to one or more of rhTPO and rhIL-11.

TPO mimetic compounds can also be used to provide autologous HSCs to a subject. Typically, this involves the steps of administering a TPO mimetic compound to a subject in need thereof to enhance expansion of the stem cell population within bone marrow and/or to mobilize the stem cells in peripheral circulation; harvesting one or more of the bone marrow stem cells or one or more of the stem cells in the peripheral circulation; and transplanting the one or more harvested stem cells back into the subject.

In addition, the stem cells obtained from harvesting according to method of the present invention described above can be cryopreserved using techniques known in the art for stem cell cryopreservation. Accordingly, using cryopreservation, the stem cells can be maintained such that once it is determined that a subject is in need of stem cell transplantation, the stem cells can be thawed and transplanted back into the subject.

The TPO mimetic compounds, including the compounds set forth in Fig. 1 and disclosed herein as well as the PEGylated forms of the compounds set forth in Fig. 1, can thus be used for, inter alia: reducing the time to engraftment following reinfusion of stem cells in a subject; reducing the incidence of delayed primary engraftment;

reducing the incidence of secondary failure of platelet production; and reducing the time of platelet and/or neutrophil engraftment following reinfusion of stem cells in a subject. These methods typically include the steps of administering a TPO mimetic compound to a subject in need thereof to enhance expansion of the stem cell population within bone marrow and/or mobilize the stem cells in peripheral circulation and then harvesting one or more of the bone marrow stem cells or the stem cells in the peripheral circulation and then transplanting the harvested stem cell back into the subject at the appropriate time, as determined by the particular needs of the subject.

The method of the invention may also be used to increase the number of stem cells from a donor subject whose cells are then used for rescue of a recipient subject who has received bone marrow ablating chemotherapy.

A. Dosage Forms and Routes of Administration

The TPO mimetic compounds useful for the present invention can be administered as pharmaceutical compositions comprising, as an active ingredient, at least one of the peptides or peptide mimetics set forth in Fig. 1 and/or disclosed herein and/or described in U.S. Patent No. 5,869,451, the entire content of which is hereby incorporated by reference, in association with a pharmaceutical carrier or diluent. The compounds can be administered by oral, pulmonary, parental (intramuscular, intraperitoneal, intravenous (IV) or subcutaneous injection), inhalation (via a fine powder formulation), transdermal, nasal, vaginal, rectal, or sublingual routes of administration and can be formulated in dosage forms appropriate for each route of administration. See, e.g., Bernstein, et al., PCT Patent Publication No. WO 93/25221; Pitt, et al., PCT Patent Publication No. WO 94/17784; and Pitt, et al., European Patent Application 613,683, each of which is incorporated herein by reference.

Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, the active compound can be admixed with at least one inert pharmaceutically acceptable carrier such as sucrose, lactose, or starch. Such dosage forms can also comprise, as is normal practice, additional substances other than inert diluents, e.g., lubricating agents such as magnesium stearate. In the case of capsules, tablets, and pills, the dosage forms may also comprise buffering agents. Tablets and pills can additionally be prepared with enteric coatings.

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups, with the elixirs containing inert diluents commonly used in the art, such as water. Besides such inert diluents, compositions can also include adjuvants, such as wetting agents, emulsifying and suspending agents, and sweetening, flavoring, and perfuming agents.

Preparations for parental administration include sterile aqueous or non-aqueous solutions, suspensions, or emulsions. Examples of non-aqueous solvents or vehicles are propylene glycol, polyethylene glycol, vegetable oils, such as olive oil and corn oil, gelatin, and injectable organic esters such as ethyl oleate. Such dosage forms may also contain adjuvants such as preserving, wetting, emulsifying, and dispersing agents. They may be sterilized by, for example, filtration through a bacteria retaining filter, by incorporating sterilizing agents into the compositions, by irradiating the compositions, or by heating the compositions. They can also be manufactured using sterile water, or some other sterile injectable medium, immediately before use.

Compositions for rectal or vaginal administration are preferably suppositories which may contain, in addition to the active substance, excipients such as cocoa butter or a suppository wax. Compositions for nasal or sublingual administration are also prepared with standard excipients well known in the art.

The compositions of the invention can also be microencapsulated by, for example, the method of Tice and Bibi (in Treatise on Controlled Drug Delivery, ed. A. Kydonieus, Marcel Dekker, New York (1992), pp. 315-339).

The composition can also be combined with, inter alia, G-CSF, SCF, IL-3 or Flt-3 and/or other agents that can enhance stem cell mobilization from bone marrow (including priming chemotherapy and integrin antagonists).

B. Dosage Amount

The quantities of a TPO mimetic compound necessary for the present invention will depend upon many different factors, including means of administration, target site, physiological state of the subject, and other medicants administered. Thus, treatment dosages should be titrated to optimize safety and efficacy. Typically, dosages used in vitro may provide useful guidance in the amounts useful for in situ administration of these reagents. Animal testing of effective doses for treatment of particular disorders will provide further predictive indication of human dosage. Various considerations are described, e.g., in Gilman, et al. (eds), Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 8th ed., Pergamon Press (1990); and Remington's Pharmaceutical Sciences, 7th Ed., Mack Publishing Co., Easton, Pa. (1985); each of which is hereby incorporated by reference.

The TPO mimetic compounds are useful for the present invention when administered at a dosage range of from about 0.001 mg to about 20 mg/kg of body weight per day. Alternatively, in some instances 0.0001mg/kg to about 10 mg/kg may also be administered. The specific dose employed is regulated by the particular condition being treated, the route of administration as well as by the judgement of the attending clinician depending upon factors such as the severity of the condition, the age and general condition of the subject, and the like.

C. Subjects and Indications

As used herein, a subject includes anyone who is a candidate for autologous stem cell or bone marrow transplantation during the course of treatment for malignant disease or as a component of gene therapy. Other possible candidates are subjects who donate stem cells or bone marrow to subjects for allogeneic transplantation for malignant disease or gene therapy.

In order to provide an acceptable probability of engraftment, a minimum number of stem cells must be harvested. Though not precisely defined, it is generally accepted that 2-3 x 10^6 $CD34^+$ cells per kg must be harvested in order to provide a reasonable chance of engraftment. Reinfusion of 5 x 10^6/kg cells appears to produce optimum results in terms of time to engraftment. This large number of cells is required because the actual number of the specific subset of $CD34^+$ cells that are capable of long term reconstitution of bone marrow is very small. As many as 20% of transplanted

patients are considered to be poor mobilizers, requiring multiple aphereses to generate sufficient cells. Although one of the most important predictors of poor stem cell mobilization is the age of the patient, heavy pretreatment with chemotherapy is also a significant factor. There are likely a large number of patients, particularly elderly patients with myeloma or NHL, who are not considered for ASCT because of the low probability of successful engraftment.

Consequently the method of the invention provides a solution to the unmet need in ASCT, i.e., the need to improve the proportion of patients who have successful and rapid engraftment. This is achieved primarily due to improvement in mobilization of stem cells, either by increasing numbers of mobilized cells or by increasing the proportion of HSCs in the mobilized CD34+ population. The method of the invention thus provides the following benefits:

1. Allows transplantation to proceed in patients who would not otherwise be considered as candidates because of the unacceptably high risk of failed engraftment;
2. Reduces the number of aphereses required to generate a minimum acceptable harvest;
3. Reduces the incidence of primary and secondary failure of engraftment by increasing the number HSCs available for transplantation; and
4. Reduces the time required for primary engraftment by increasing the number of committed precursors of the important hemopoietic lineages.

In accordance with the established effect of TPO on HSCs, the TPO mimetic compounds of the invention may have the following clinical benefits in stem cell transplantation:

- Improvement of apheresis yields: Numerous studies have suggested that the number of reinfused $CD34^+$ stem cells is an important factor in determining the time to engraftment. As demonstrated with TPO, the addition of a TPO mimetic compound may increase mobilization of $CD34^+$ cells as an adjunct to conventional mobilization regimens of G-CSF and chemotherapy. The primary benefit would be to improve the

prospect for rapid and subsequent long term engraftment. Reducing the number of aphereses required to generate an acceptable number of cells would reduce cost and patient inconvenience. Improvement in apheresis yields would be of particular benefit in patients with risk factors for low mobilization (age and heavy pretreatment). Such patients may otherwise not be candidates for ASCT.

- Improvement of the engraftment potential of apheresed cells: Long term engraftment following myeloablative therapy is produced by a small fraction of the $CD34^+$ cell population (most likely within the $CD34^+CD38^-Lin^-$ population). Because they are so rare, large numbers of $CD34^+$ cells are required to provide effective engraftment (2-5 $x10^6$/kg). G-CSF does not affect the proportions of different subtypes of $CD34^+$ cells and is simply used as an agent that can increase the number of these cells in peripheral blood prior to harvest. Priming chemotherapy may actually be toxic to such cells. However TPO is increasingly widely recognized as an agent that can increase the self-renewal of the most primitive stem cells, and thus capable of long-term hemopoietic reconstitution. A similar effect of a TPO mimetic compound may therefore increase the proportion of the $CD34^+$ cell population that can contribute to long-term engraftment and thus reduce the risk of failure of engraftment. TPO may also increase the numbers of stem cells committed to the megakaryocytic lineage thus producing earlier independence from platelet transfusions, i.e., reduced time to engraftment.

The two beneficial effects described above may be additive or synergistic, leading to greater reduction in time to engraftment than might be seen with agents that only increase mobilization of stem cells.

The use of a TPO mimetic compound of the invention would likely require only a small number of doses given, e.g., intravenously or subcutaneously, prior to

apheresis. Such a dosing regimen would minimize the risk of significant antigenicity, which is already predicted to be low due to the use of a pegylated product.

The TPO mimetic compound of the invention is first administered to normal volunteers:

1. To establish the effect of the TPO mimetic compound on peripheral blood $CD34^+$ cell populations, platelet counts and other hematological parameters;
2. To establish the preliminary safety profile of the TPO mimetic compound in terms of dose limiting toxicity and high frequency adverse events;
3. To determine the most appropriate dose, dose regimen and dose timing of pre-apheresis dosing with the TPO mimetic compound; and
4. To determine the pharmacokinetic profile of the TPO mimetic compound in humans.
5. To generate preliminary comparative information on the effects of a TPO mimetic compound of the invention and G-CSF on the multilineage potential of peripheral blood stem cells.

Normal human volunteers, which are the most appropriate population for evaluation of pharmacokinetics and initial safety profile, provide the clearest understanding of the effects of the TPO mimetic compound on HSCs because of the absence of the background effects of chemotherapy and disease.

The study will be a single blind, dose rising study in which normal human volunteers receive a single intravenous dose of the TPO mimetic compound of the invention, given as a one hour infusion. The starting dose will be 15 ug/kg. Successive dose cohorts will receive 25, 50, 100 and 200 ug/kg. Four subjects will be enrolled in each cohort, three of whom will receive active therapy and one whom will receive placebo. Each subject will be observed at regular (15 minute) intervals during the infusion and will remain as an inpatient for 24 hours for close safety monitoring and pharmacokinetic sampling. Further outpatient follow up for safety, pharmacokinetic and pharmacodynamic evaluation will occur on days 2, 4, 7, 14, 21 and 28. Each successive dose cohort will be treated two weeks after the previous group.

When a dose is reached at which evidence of pharmacodynamic effect (defined as a 50% increase in platelet count relative to the pretreatment value) is observed in 2/3 of the actively treated subjects dosing at that level will be extended to include a further four subjects (3/1 active/placebo). If the efficacy is confirmed, one further dose cohort of six subjects (4/2 active/placebo) will be enrolled to provide further confirmation of the pharmacodynamic effect. If the pharmacodynamic effect is only observed at the highest planned dose, a further dose increase will be considered, assuming that no evidence of toxicity has been observed.

If a safety /tolerability event that is possibly or probably related to study medication occurs in a single actively treated subject at any dose four further subjects (3/1 active/placebo) will be enrolled at that dose to determine if a dose limiting toxicity has been identified.

Blood samples will be taken for measurement of drug levels 30 minutes after the beginning of the infusion, at the end of the infusion and at the following times after the end of the infusion: 5 minutes, 15 minutes, 30 minutes, 1, 2, 4, 8, 12, 24, 48, 96 hours and 7 and 14, 21 and 28 days. Compound levels will be measured using either a cell based bioassay or by ELISA.

For comparison, a single dose of G-CSF will be administered to three subjects to measure the effects on CD34+ cells.

The effect of the TPO mimetic compound on peripheral blood $CD34^+$ counts, if any will be delayed for several days (3-7 if the effect is similar to thrombopoietin). Furthermore because of the unknown impact of the TPO mimetic compound pharmacokinetics on the PD profile it is not certain when the maximal effect of a single dose will be seen. It is the timing of the maximal effect that will determine the interval between the TPO mimetic compound dosing and harvest of $CD34^+$ cells in subsequent patient studies. A good correlation between peripheral blood $CD34^+$ counts and the yield in subsequent harvesting has been demonstrated suggesting that this approach is reasonable. It may be appropriate to ensure that the TPO mimetic compound invention is given prior to G-CSF to enable an expansion of the HSC population within bone marrow followed by mobilization of the expanded population. Most studies that use G-CSF to stimulate mobilization give the drug for five days with the harvest stated

towards the end of the dosing period. It is possible that the pharmacodynamic profile of the TPO mimetic compound will require it to be dosed some days prior to G-CSF.

The impact of the TPO mimetic compound on the number of self-renewing HSCs in the mobilized population could provide an increase in self renewal capacity which could lead to successful engraftment with lower numbers of mobilized cells, greater ease of performing tandem transplants and the possibility that the TPO mimetic compound could eventually replace G-CSF as the standard mobilizing agent.

This aspect of the clinical pharmacology of the TPO mimetic compound can be addressed by measuring the self renewal capacity of $CD34^+$ populations from the normal volunteers study, both in *in vitro* studies of the ability to sustain long term colony formation (the LTC-IC culture) and by performing SCID/NOD mouse repopulation assays in which the mobilized cells are infused into lethally irradiated SCID/NOD mice. Preliminary calculations indicate that performing such studies should be feasible with the $CD34^+$ cells that are contained in 30-50 mls of blood, provided that the $CD34^+$ count has risen to approximately $15x10^3$/ml.

The assumptions underlying this statement are outlined below:

1. PBMCs from normal subjects will be obtained by Ficoll/Hypaque separation and then Lin+ cells will be removed by negative selection. CD34+ CD38- subfractions of this enriched population will then be isolated by FACS and administered to SCID/NOD mice. Mice will also receive accessory cells and growth factors to permit the use of lower numbers of CD34+CD38-Lin- cells per mouse (Bonnet et al., Bone, Marrow Transplantation, 23:203-209 (1999)). Alternatively, the original PBMC population will be used without further purification to provide both repopulating and accessory cells.
2. The primary endpoint for this assay will be survival of the recipient mice. However Southern Blot analyses will also be performed to detect human DNA in the recipient mice. If possible, detection of human progenitor cells will be determined by human selective long term marrow cultures and/or flow cytometry with human specific MAbs.

3. Each subject will provide enough blood to test four CD34+CD38-Lin- cell doses (250, 500, 1000 and 2000 cells/mouse). Each cell dose will be given to 5 mice. With these design assumptions approximately $1.9x10^4$ CD34+CD38-Lin- cells will be needed from each subject. If additional in vitro colony forming studies are performed, more cells will be needed.

4. Each normal subject will provide this blood sample only once and only when the CD34+ cell count in peripheral blood has reached 15x103/ml. CD34+CD38-Lin- population represents 5-8% of CD34+ population (Gallacher et al., Blood, 95:2813-2820 (2000)). Studies with TPO in normal volunteers indicated that $16x10^3$ CD34+ cells/ml were seen in the peripheral blood.

5. 30 mls of blood will be required from each subject to yield 2.25-3.6x104 cells.

6. It will not be possible to perform these studies in placebo treated subjects due to low levels of CD34+ cells ($<3x10^3$/ml). For comparison, similar quantities of cells will be taken from subjects treated with G-CSF. Equal numbers of cells will be infused into the mice.

7. The validity of these assumptions is tested with independent data. Approximately 1 in $6x10^6$ PBMCs is capable of repopulating a SCID/NOD mouse (Wang et al., Blood, 89:3919-3924 (1997)). Of this population, the CD34+ population is 0.13-0.39% and 5-8% of this subset is CD34+CD38-Lin- (Tichelli et al., Br. J. Hematol., 106:152-158 (1999)). This represents 390-1872 cells from the original $6x10^6$ PBMCs. In a separate study the incidence of SCID/NOD repopulating cells in the CD34+CD38-Lin- population has been demonstrated to be 1 in 617 (Bhatia et al., PNAS, 94:5320-5325 (1997)). This number is consistent with the extrapolation to the incidence in unselected cells.

8. If cell number becomes limiting, the highest cell dose cohort of mice will be dropped.

$CD34^+$ cells taken from volunteers who are given G-CSF will be used as a control for these studies.

The proposed normal volunteer study will provide the database required to determine the design of patient studies in terms of dose, dose regimen and dose timing as well as strong pharmacodynamic evidence that predicts clinical efficacy. The next phase of the clinical pharmacology program will seek to reproduce the observed effects in patients scheduled for stem cell transplantation as well as providing translational data that will demonstrate a link between the pharmacodynamic endpoints described above and the clinical endpoints required for regulatory approval. The mimetic compound of the present invention is then administered to patients in need:

1. To explore the risk to benefit profile of the TPO mimetic compound in different populations of patients who are candidates for autologous stem cell transplantation; and
2. To obtain preliminary evidence of the likely effect of the TPO mimetic compound on apheresis yields of peripheral blood $CD34^+$ stem cells and post engraftment outcomes.

The first patient study will again be a single dose, dose rising design (assuming that there is no reason to give the dose of the TPO mimetic compound as a divided dose). Dosing of the TPO mimetic compound will be introduced into a standard mobilization regimen, with the dose interval between dosing and harvest predicted from the volunteer study. The same pharmacodynamic endpoints will be evaluated in this study as in the previous study, but data on apheresis yields, number of aphereses and subsequent rates and times of engraftment will also be obtained. Dosing will be given via single use 10-20 mg vial containing lyophilized powder as a single dose by intravenous bolus administration before apheresis and after reinfusion of harvested cells. A subcutaneous dosing bioequivalent can be administered with intravenous dosing. It is expected that the dose would be between about 10-300 μg/kg.

A key aspect of this study will be to explore the risk to benefit of the TPO mimetic compound in different patient populations. An increasing number of patients are receiving high dose, myeloablative therapy with ASCT relatively early in the course of their disease. Such patients often have relatively normal bone marrow and, particularly if they are young, are likely to mobilize acceptable numbers of CD34+ cells

with consequent high likelihood of rapid engraftment. In this population, the potential impact of an additional agent to enhance mobilization may be limited but could be manifest as even more rapid engraftment with reservation of harvested cells for tandem transplant. Nevertheless, this population, which most closely resembles the normal population at least in terms of bone marrow responsiveness, is an important translational group for the development of the TPO mimetic compound.

Patients who become candidates for ASCT after multiple previous courses of therapy often have greater difficulty in generating enough $CD34^+$ cells for an adequate harvest. Consequently, many of these patients require prolonged apheresis schedules and a higher incidence of delayed or failed engraftment. A proportion of these patients are not able to undergo autologous transplant and must instead resort to allogeneic transplant with increased risk of post transplant complications. It is this population in which an additional mobilization agent may be of great benefit.

Consequently, the first patient study will enroll patients from both categories. The data from the 'good mobilizers' will be used as a benchmark to determine the impact of the TPO mimetic compound on the 'poor mobilizers'. A non-treated group, receiving only standard of care will be included.

The pharmacodynamic endpoints outlined above will provide a robust surrogate of the likely clinical benefit of the TPO mimetic compound in ASCT.

Definitive studies will be conducted as parallel group, double blind, placebo controlled studies. Once randomization has occurred clinical decisions about transplantation will be made according to predefined rules and accepted clinical practice.

The primary endpoint for the studies will be mean time to engraftment following re-infusion of harvested cells. Time to engraftment will be defined as number of days until platelet count is maintained above $20x10^9/L$ without transfusion support for a period of 7 days.

Secondary endpoints will include:

1. Time to neutrophil engraftment (defined as neutrophil count maintained above $0.5x10^9/L$);

2. Time to platelet count >$50x10^9$/L (maintained for 7 days without transfusion support);
3. Proportion of patients with delayed platelet engraftment;
4. Proportion of patients with secondary failure of platelet engraftment;
5. Proportion of patients who fail to generate minimum harvest necessary for transplantation;
6. $CD34^+$ harvest ($CD34^+$ cells/kg);
7. Number of aphereses required for harvest; and
8. Number of platelet transfusions.

A key factor in study design will be selection of the target population. Published data indicates that the number of $CD34^+$ cells harvested is a major determinant of subsequent engraftment kinetics and will therefore directly impact the primary endpoint of the studies. The key demographic features that will influence the ability to mobilize $CD34^+$ cells is the amount of pretreatment and patient age. A number of issues must be considered:

1. If a poor mobilizing population is selected there will be the greatest opportunity to detect an improvement in engraftment rates but the ability of the bone marrow to respond to the TPO mimetic compound may be so compromised that no response is possible;
2. If a high mobilizing population is selected the ability to detect a response over background therapy may be limited due to the fact that optimal numbers of self renewing HSCs will be re-infused regardless of the addition of the TPO mimetic compound;
3. The intrinsic effect of the TPO mimetic compound of the invention to increase mobilization may prevent accurate definition of good or poor mobilizers;
4. The ability to detect the effect on engraftment of an increase in self renewing HSCs may only be seen in patients in whom the number of these cells is a limiting factor in engraftment kinetics.

On the basis of these issues it is important that the study population for these studies is limited in the number of patients at the extremes of the mobilization range. At either extreme it may be difficult to demonstrate the efficacy of the compound. This can be achieved by excluding some patient groups that are highly likely to contribute to extreme values of mobilization (for example patients receiving first line therapy, patients with myelodysplasia and/or low marrow reserve) and also by ensuring that the sample size is determined by patients who reach a predefined range of $CD34^+$ harvest size (i.e., randomized patients who failed to meet these criteria would be replaced)

If a design of this type is followed, the majority of the patients who would contribute to the primary endpoint in the placebo group would have $CD34^+$ yield falling into the following categories in the ratio 2:3:1 respectively:

$<2.0x10^6$/kg (median time to engraftment =17 days)

2-$5x10^6$/kg (median time to engraftment =12 days)

$>5x10^6$/kg. (median time to engraftment = 10 days).

In a population of this type, the expected median time to engraftment would be 13-14 days. If the effect of the TPO mimetic compound on yield of $CD34^+$ cells was to alter the proportions of the different harvest categories from 2:3:1 to 1:2:3, this change alone would result in a reduction in the median time to engraftment of 1.66 days. If an improved time to engraftment, within each category caused by increased numbers of self-renewing HSCs, is superimposed on this such that the median time to engraftment improves by 5 days in the lowest yield group (i.e., they behave like the middle yield group) and 2 days in the middle group (i.e., they behave like the high yield group), the additional reduction in median time to engraftment would be 1.66 days. No effect on time to engraftment is assumed for the high yield group. Collectively, the impact of the TPO mimetic compound treatment on median time to platelet engraftment, for the purposes of calculating a sample size, would be 3 days. To enable the maximum opportunity to define the clinical benefit of the TPO mimetic compound a relatively low threshold for the minimum harvest required to allow myeloablation to proceed should be set.

The ability to demonstrate efficacy of the TPO mimetic compound in ASCT is relatively straightforward because the observation of increased numbers of $CD34^+$ stem cell in the peripheral blood of normal volunteers treated with single doses will suffice.

The first human study will therefore demonstrate a biologically relevant effect. Several studies have identified the level of $CD34^+$ cells in peripheral blood as an important predictor of subsequent apheresis yield. However the effect on stem cell mobilization in combination with G-CSF will not be established until the first patient study is completed. It will be more difficult to establish that the mobilized $CD34^+$ cells contain increased numbers of stem cells, in part because it is difficult to measure the low levels of HSCs in unstimulated patients. However since G-CSF is reported not to affect the proportion of HSCs in the $CD34^+$ population, it may be possible to infer some effect of the TPO mimetic compound on the number of self renewing HSCs within the mobilized cell population by comparison to cells mobilized with G-CSF.

The most important predictor of success will be apheresis yield. The number of reinfused cells is an important predictor of the subsequent time to engraftment. Consequently the proportion of patients with clinically acceptable or high yields will be a major determinant of the likely impact on time to engraftment and the proportion of patients with delayed or failed engraftment.

It is expected that the TPO mimetic compound is as good as or superior to G-CSF in mobilizing stem cells and that the TPO mimetic compound provides improved quality of the mobilized stem cell population.

A single blind study to evaluate the effect of the TPO mimetic compound on mobilization of peripheral blood CD34+ stem cells when added to standard mobilization regimens in patients scheduled for myeloablative chemotherapy with autologous stem cell transplantation.

To determine the effect of the TPO mimetic compound on the mobilization of stem cells prior to apheresis, a single dose, dose rising study using doses proven to mobilize CD34+ cells in normal volunteers will be conducted. Each dose cohort will contain six patients receiving active medications and two receiving G-CSF background therapy only. Each cohort will be divided into two groups of 3 active and 1 placebo patient. One group will be patients receiving autologous SCT as first line therapy whereas the other will be heavily pretreated patients receiving autologous SCT as salvage therapy. When a dose is reached that produces an increased yield of CD34+ cells relative the placebo patient (effect size to be defined) and to historical controls for the effect of G-CSF on stem cell mobilization, eight additional patients (per subcohort) will be recruited at that dose to solidify the evidence of efficacy and to explore

additional pre and post transplant endpoints (to include number of aphereses required to yield 3 x10^6 cells/kg, the proportion of patients who attain an adequate harvest and the time to post transplantation neutrophil recovery and platelet transfusion independence). Further dose increases will continue as per the original randomization schedule. If one sub-cohort reaches an efficacy plateau or dose limiting toxicity, the remaining sub-cohort will continue in dose escalation. At the time of apheresis, a sample of apheresed cells will be obtained for study of the multipotential capacity of the harvested cells (assuming that the size of the harvest is not limiting). After meeting screening criteria and collection of baseline blood samples, the patient will be receive a single dose of study medication given by intravenous infusion over a period of 60 minutes. Follow up visits will occur every 48 hours until stem cell harvest is complete. Stem cell harvest will be deemed to have failed if 10 aphereses have failed to yield sufficient cells for successful engraftment (minimum 2 x10^6/kg). The patient will then continue with myeloablative chemotherapy, reinfusion of stem cells and follow up with appropriate supportive care according to the protocol defined for the patients tumor. Data on engraftment will be abstracted from the source documents according to predefined specifications

Samples will be taken for pharmacokinetic sampling at each study visit. Compound levels will be measured using an ELISA.

It is believed that the administration of the TPO mimetic compound in accordance with the method of the invention will provide a number of advantages, including, inter alia:

- Reduction in median time to platelet engraftment (defined as platelet count >20x10^9/L) of 3 days when added to standard therapy. Reduction is 1 day when used instead of standard therapy.
- Reduction in the proportion of patients with delayed time to platelet engraftment from 40% to 10%.
- Increase in the proportion of patients who attain primary platelet recovery (defined as patients who maintain a platelet count >50,000 for 7 days) from 60% to 85%.
- Reduction in number of platelet transfusions required (from a median of 5 to a median of 3).
- Reduction in median time to ANC>0.5 x 10^9/L of 1 day.

- Reduction in the proportion of patients who fail to meet minimum stem cell harvests (3 x10^6/kg) for transplantation (from 35% to 5%) when used in combination with G-CSF.
- Increase in the yield of CD34+ cells when used in combination with G-CSF (4 x 10^6/kg vs 1 x10^6/kg).
- Reduction in the number of harvests required to yield sufficient cells to support transplantation when used in combination with G-CSF (from a median of 3 to a median of 1).

Convenient single dose therapy to improve the efficiency of stem cell transplantation, to permit more aggressive treatment of solid tumors, myeloma and lymphoma and to increase the number of candidates for stem cell transplantation.

Although only particular embodiments of the invention are specifically described above, it will be appreciated that modifications and variations of the invention are possible without departing from the spirit and intended scope of the invention.

What is claimed is:

1. A method of increasing hematopoietic stem cell production in a subject comprising a step of administering a TPO mimetic compound to said subject.

2. A method of providing hematopoietic stem cells to a subject comprising the steps of:

administering a TPO mimetic compound to a subject to enhance expansion of a stem cell population within bone marrow and/or mobilize stem cells in peripheral circulation;

harvesting one or more of the bone marrow stem cells or the stem cells in the peripheral circulation; and

transplanting the harvested stem cells into the subject.

3. The method of claims 1 and 2, wherein the subject is a human.

4. The method of claim 2, wherein the one or more stem cells are cryopreserved after harvesting.

5. The method of claim 4, wherein the one or more cryopreserved stem cells are thawed and determined to be viable prior to transplanting the stem cells into the subject.

6. The method of claim 4, wherein the one or more stem cells are transplanted into the subject when the subject is in need of such transplantation.

7. The method of claims 1 and 2, wherein the TPO mimetic compound has reduced immunogenicity relative to one or more of rhTPO and rhIL-11.

8. The method of claims 1 and 2 wherein the TPO mimetic compound has an improved PK profile relative to one or more of rhTPO and rhIL-11.

9. A method of reducing a time to engraftment following reinfusion of stem cells in a subject comprising the steps of:

administering a TPO mimetic compound to the subject;

enhancing the expansion of the stem cell population within bone marrow and/or mobilizing the stem cells in peripheral circulation; and

harvesting one or more of the bone marrow stem cells or one or more of the stem cells in the peripheral circulation; and

transplanting the one or more harvested stem cells into the subject.

10. A method of reducing the incidence of delayed primary engraftment comprising the steps of:

administering a TPO mimetic compound to the subject;

enhancing the expansion of the stem cell population within bone marrow and/or mobilizing the stem cells in peripheral circulation; and

harvesting one or more of the bone marrow stem cells or one or more of the stem cells in the peripheral circulation; and

transplanting the one or more harvested stem cells into the subject.

11. A method of reducing the incidence of secondary failure of platelet production comprising the steps of:

administering a TPO mimetic compound to the subject;

enhancing the expansion of the stem cell population within bone marrow and/or mobilizing the stem cells in peripheral circulation; and

harvesting one or more of the bone marrow stem cells or one or more of the stem cells in the peripheral circulation; and

transplanting the one or more harvested stem cells into the subject.

12. A method of reducing the time of platelet and/or neutrophil engraftment following reinfusion of stem cells in a subject comprising the steps of:

administering a TPO mimetic compound to the subject;

enhancing the expansion of the stem cell population within bone marrow and/or mobilizing the stem cells in peripheral circulation; and

harvesting one or more of the bone marrow stem cells or one or more of the stem cells in the peripheral circulation; and

transplanting the one or more harvested stem cells into the subject.